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Short communication

Polyelectrolyte complex materials from chitosan and gellan gum

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ABSTRACT

The formation of polyelectrolyte complexation (PEC) films by dipping free-standing films of either gellan gum (GG) or chitosan (CH) into solutions of opposite charge is reported. It was shown that the composition and mechanical characteristics depend on pH, and the order in which the biopolymers were added, i.e., dipping CH into GG, or dipping GG into CH. Toughness, tensile strength, extensibility and density values of PEC films prepared by immersing CH films into GG solutions increased with increasing CH:GG ratio. This was not observed for PEC films prepared by dipping GG into CH. The observed behaviour is attributed to the difference in their film compositions, i.e., films prepared by dipping GG into CH solution consist of an inner CH layer and two outer GG layers. In contrast, GG appears to diffuse into the CH layer for films prepared by dipping CH into GG.

1. Introduction

Formation of films through polyelectrolyte complexation (PEC) or electrostatic self-assembly involves dipping a charged substrate into dilute solutions of opposite charge (Kujawa, Moraille, Sanchez, Badia, & Winnik, 2005). Potential application areas for PEC films include nanofiltration (Jin, Toutianoush & Tieke, 2003; Miller & Bruening, 2004), pervaporation (Krasemann & Tieke, 1998), coating (van den Beucken et al., 2006), tissue engineering (Tang, Wang, Podsiadlo, & Kotov, 2006), and encapsulation (Köhler & Sukhorukov, 2007). A number of synthetic and biopolymers have been successfully employed in polyelectrolyte complexation, such as poly(styrene sulfonate)/poly(allylamine) (Ladam, Schaad, Voegel, Schaaf, Decher, & Cuisinier, 2000), and poly(L-lysine)/alginate (Elbert, Herbert, & Hubbell, 1999).

Layer-by-layer (LbL) self-assembly is a well-known method for PEC film formation has been studied for many years (Decher, 1997). LbL involves sequentially dipping and washing of a substrate in dilute solutions of cationic and anionic polymers. The resulting complexes have many interesting properties, but are limited to thin films by the fabrication process which adds 1–2 nm in thickness per cycle of dipping and washing.

Natural polysaccharides such as chitosan (CH) and gellan gum (GG) are suitable materials for polyelectrolyte complexation. Chitosan is a cationic polyelectrolyte derived from chitin, the second most abundant naturally occurring polysaccharide (Brine, Sandford, & Zikakis, 1982). Chitin is found in crustacean shells, insects, and also synthesized from certain plants (Agullo, Rodriguez, Ramos, & Albertengo, 2003; Kurita, 2006). Gellan gum is an anionic polysaccharide produced by *Sphingomonas elodea* bacteria (Giavasis, Harvey, & McNeil, 2000). Chitosan and gellan gum are attractive polyelectrolytes due to their biocompatibility and biodegradability. For example, GG is US FDA and European Union (E 415) approved for food usage, and is also emerging as a scaffold material for tissue engineering application (Smith, Shelton, Perrie, & Harris, 2007). CH is used in scaffolds as it promotes cell adhesion, proliferation and differentiation (Francis Suh & Matthew, 2000; Ge et al., 2006), and increases the wound healing rate (Aoyagi, Onishi, & Machida, 2007; Xu, Ma, Shi, Gao, & Han, 2007). To date, polyelectrolyte complexation involving CH and GG has focused on the development of capsules and fibers (Amaike, Senoo, & Yamamoto, 1998; Granero, Razal, Wallace, & Panhuis, 2009).

In this paper, we describe a new approach to forming PEC films using the oppositely charged biopolymers chitosan and gellan gum. PEC films are formed by immersion of biopolymer films in a biopolymer solution of opposite charge, before turning over, and immersing again. The mechanical, swelling and water vapour permeability properties of these PEC films are investigated.

2. Experimental

2.1. Materials and film formation

Chitosan (CH; medium molecular weight, $M_w \approx 1.9–3.1 \times 10^5$ g/mol, 75% degree of deacetylation (DD), viscosity ≈ 453 mPa s (1% (w/v) chitosan in 1% acetic acid), product number 448877 – lot number 07918TE), and glycerin were obtained from Sigma Aldrich. Gellan gum (GG; low acyl, $M_w \approx 2–3 \times 10^5$ Da, lot number

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7K 1383A) was a gift from CP Kelco, USA. According to the manufacturer's specification, all acyl groups have been removed from the glucose residue for this form of gellan gum. All materials were used as received.

CH (2% (w/v)) and glycerin (0.6% (w/v)) were dissolved in an acetic acid (0.08 M) solution under continuous stirring for 2 h at 70 °C. GG (1% (w/v)) and glycerin (0.5% (w/v)) were dissolved in Milli-Q water (18.2 MΩ cm) under continuous stirring for 2 h at 70 °C. The pH values of these as-prepared CH and GG solutions were pH ~5.6 and pH ~5.4, respectively. CH and GG solutions with pH ~1.8 (CH) and pH ~12 (GG) were prepared from the as-prepared solutions through addition of concentrated acetic acid (glacial, 17 M) and sodium hydroxide (1 M), respectively. These solutions with adjusted pH are hereafter referred to as CH(pH) and GG(pH). Free-standing films of CH, CH(pH), GG and GG (pH) were prepared by evaporative casting of solutions onto an acrylic substrate and dried under controlled ambient conditions ~21 °C, 50% relative humidity (RH) for 48 h. The films were then peeled off to yield uniform free-standing single biopolymer (SB) films.

Polyelectrolyte complex (PEC) films were prepared by immersing the SB films into biopolymer solution of opposite charge for 30 s on each side, i.e., they were laid flat for 30 s, before turning over, and laid flat for another further 30 s. The resulting films were dried on a custom build rack under controlled ambient conditions for 48 h. CH films were immersed in GG solutions at 70 °C, while GG films were immersed in CH solutions at 25 °C.

2.2. Characterisation

UV–vis absorption and transmission spectra were recorded on a Varian Cary 500 scan spectrophotometer at 21 °C. Films were preconditioned for 2 days under controlled conditions of 21 °C and 50% RH. All testing of films was carried out under these controlled conditions. Stress–strain measurements were obtained using an Instron Universal Testing Machine (model 5566) with ±10 kN grips and cross-head speed 20 mm min^{-1}. Films were cut to 2.5 cm × 10 cm, and film thickness was measured using a hand-held micrometer (Mitutoyo). Young's modulus, tensile strength, toughness, and elongation were calculated from the slope of the linear part of the stress–strain curve, the maximum stress, the area under the stress–strain curve, and percentage elongation at break, respectively.

Optical microscopy and image analysis (LEICA DFC290 optical microscope coupled to LEICA Application Suite software) were used to image the SB and PEC films.

The pH values of solutions and films were determined using a pH meter (Metrohm 826 pH mobile). The pH of films were determined using a flat-membrane glass electrode, and slight wetting of the surface prior to measurement.

Elemental analysis (C, H, N) of films and starting materials were carried out by the University of Otago, New Zealand to determine the elemental composition. This information is converted into one matrix (M_s) for the starting materials, and several arrays, one for each of the films. The film's composition in terms of percentage of each of the biopolymers is then obtained from the matrix product of the films array and the inverse of the matrix M_s.

Swelling was measured by weighing dried films (L_{dry}) prior to immersion into solutions of pH 1, pH 7 and pH 12 at 21 ± 0.5 °C. After 24 h, the films were removed, wiped gently with a tissue to remove surface solution and weighed (L_{wet}). Swelling was determined from the equilibrium-swelling ratio, $(L_{\text{wet}} - L_{\text{dry}})/L_{\text{dry}}$. The pH values of films and solutions were determined using a Metrohm 826 pH meter.

Water vapor analysis of the films was performed using a modified ASTM International standard method (Wu, Fisher, Foo, Queen, & Gaylor, 1995). Each sample is fixed on the circular opening of a permeation bottle (d = 1.5 cm, height = 5.0 cm) with effective transfer area (A = 1.33 cm^2), and placed in a temperature and humidity controlled chamber (Thermoline Scientific model TRH-150) at 21 °C, 50 ± 5% RH. The weight of the bottle with water is then monitored as a function of time. The water vapor transmission rates (WVTRs), water vapor permeability (WVP) and permeance (PR) values were obtained using Eqs. (1)–(3) (Gontard, Guilbert, & Cuq, 1993).

$$\text{WVTR} = \frac{m}{\Delta tA} \tag{1}$$

$$\text{WVP} = \frac{m}{\Delta t \Delta P} \tag{2}$$

$$\text{PR} = \frac{m}{\Delta tA\Delta P} \tag{3}$$

where $m/\Delta t$ is the amount of water lost per unit time of transfer, X is the film thickness, A is the area exposed to water transfer (m^2), and ΔP is the water vapor pressure difference between the permeation bottle and humidity chamber.

3. Results and discussion

3.1. Single biopolymer films

Large free-standing films (10 cm × 20 cm) of CH and GG were successfully prepared using evaporative casting (Fig. 1). Addition of glycerin leads to mechanical weakening, but makes the films less brittle and easier to handle (Mat Amin, Matic, Walker, Wilson, Poon, & Panhuis, submitted for publication), in particular for GG (results not shown). The resulting films were clear with a 70% transmittance for wavelength range 400–800 nm. A comparison between films prepared using solutions of CH and GG revealed that CH films have higher toughness and extensibility but lower Young's modulus and tensile strength compared to GG films (Fig. 2 and Table 1). In addition, CH films were less susceptible to swelling, while the water vapor transmission rate (WVTR) and permeance were similar to that of GG (Table 2).

3.2. pH adjusted single biopolymer films

It is assumed that changes in the pH of the solutions lead to changes in the ionic nature of the solutions. For example, most of the CH's amine groups will be protonated in a CH(pH) solution (pH ~1.8), and most of the GG's carboxy groups will be deprotonated in a GG(pH) solution (pH ~12). The effect of solution pH on the film's mechanical properties is profound. For example, films from CH(pH) solutions with pH ~1.8 exhibit a 4.8 fold increase in Young's modulus compared to films prepared using CH solutions with pH ~5.6. Smaller increases were observed for toughness (1.9 fold), tensile strength (1.7 fold) and extensibility (1.4 fold). In contrast, films prepared using GG(pH) solutions (pH ~12) resulted in large increases in toughness (6.5 fold) and extensibility (2.8 fold), but at a cost of reduced Young's modulus (1.9 fold decrease) and tensile strength (1.3 fold) compared to films prepared using GG solutions (pH ~5.4). The increase in tensile strength values for the CH material is likely related to the observed increase in density, from 655 ± 71 kg m^{-3} to 1372 ± 40 kg m^{-3}. As previously suggested, this would result in a closer packing of the biopolymers, which may inhibit sliding of the chains under stress in the load direction (Kuboe, Tonegawa, Ohkawa, & Yamamoto, 2003). The opposite occurs for GG films, with a decrease in both density and tensile strength.

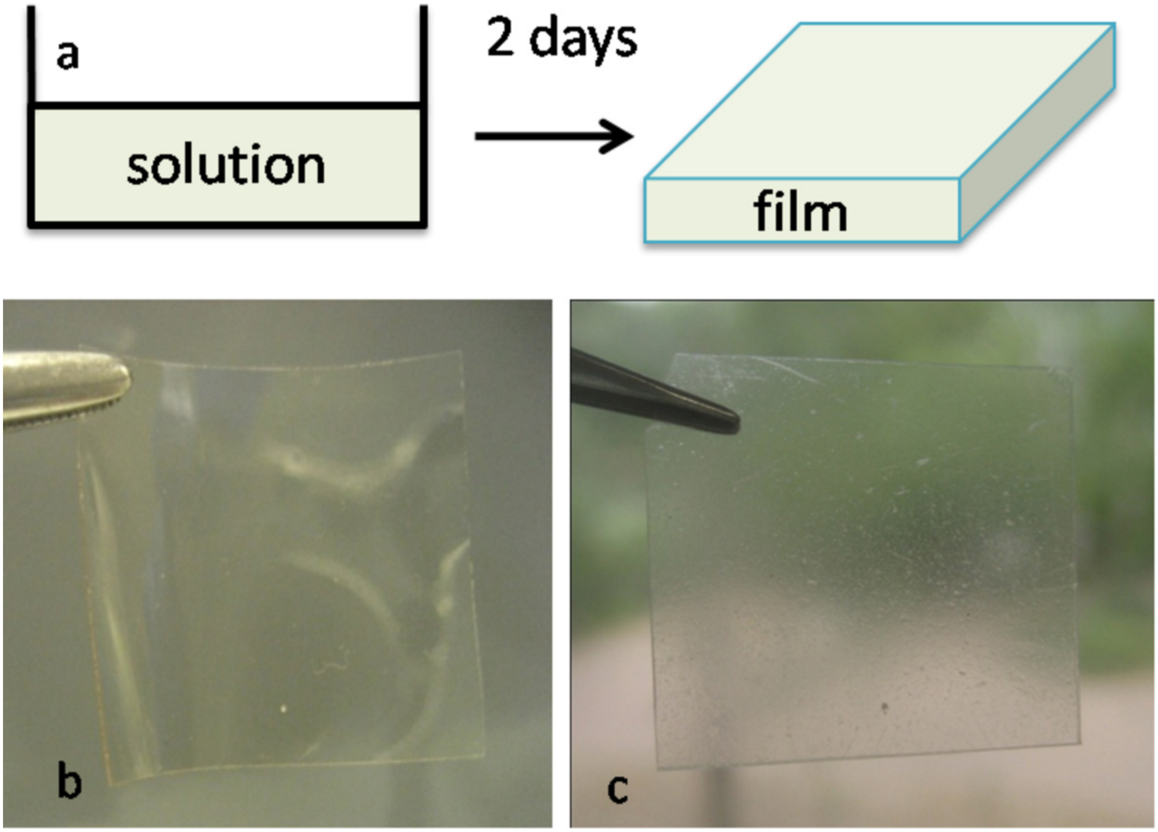

Fig. 1. Single-polymer films. (a) Schematic of the preparation process. (b) and (c) Photographs of CH and GG free-standing films (2 cm × 2 cm), respectively.

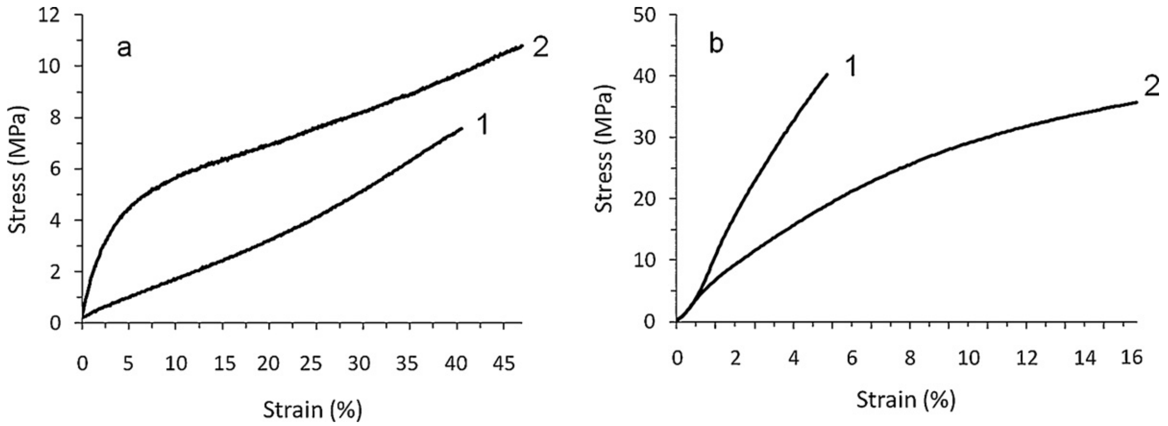

Fig. 2. Stress–strain curves for typical single-polymer films. (a) CH (1) and CH(pH) (2) films, and (b) GG (1) and GG(pH) (2) films.

Table 1
Summary of composition of the films determined using elemental analysis, CH:GG ratios, tensile strength (TS), Young's modulus (YM), toughness (*T*) and elongation at break (*E*) values.

Film	C (at.%)	H (at.%)	N (at.%)	CH (%)	GG (%)	CH:GG	TS (MPa)	YM (MPa)	*T* (J/g)	*E* (%)
CH	0.75	0.12	0.13	100	0	–	15 ± 6	25 ± 7	1.27 ± 0.01	32 ± 2
CH^{into}GG	0.78	0.13	0.09	60	40	1.5	12 ± 2	103 ± 4	2.72 ± 0.28	19 ± 1
CH^{into}GG(pH)	0.80	0.14	0.06	32	68	0.47	26 ± 6	130 ± 34	7.20 ± 2.58	45 ± 5
CH(pH)	0.75	0.12	0.13	100	0	–	10 ± 1	120 ± 42	2.44 ± 0.21	45 ± 2
CH(pH)^{into}GG	0.80	0.14	0.06	32	68	1.0	16 ± 1	322 ± 177	3.47 ± 1.07	20 ± 1
CH(pH)^{into}GG(pH)	0.79	0.13	0.08	51	49	0.56	23 ± 4	202 ± 38	4.11 ± 1.36	26 ± 3
GG	0.87	0.13	0	0	100	–	42 ± 5	1097 ± 163	0.68 ± 0.03	5 ± 1
GG^{into}CH	0.83	0.14	0.03	18	82	0.22	20 ± 8	282 ± 123	0.76 ± 0.31	12 ± 1
GG^{into}CH(pH)	0.85	0.13	0.01	7	93	0.08	27 ± 2	1345 ± 120	0.81 ± 0.81	3 ± 1
GG(pH)	0.87	0.13	0	0	100	–	32 ± 8	576 ± 136	4.45 ± 1.19	15 ± 4
GG(pH)^{into}CH	0.82	0.14	0.04	21	79	0.27	30 ± 5	329 ± 81	5.12 ± 0.12	22 ± 3
GG(pH)^{into}CH(pH)	0.85	0.14	0.01	5	95	0.05	21 ± 3	170 ± 37	2.05 ± 1.37	19 ± 2

Table 2
Summary of the water vapor transmission rates (WVTR), water vapor permeability (WVP), permeance (PR) values, and swelling upon immersion into solutions of pH 1, pH 7 and pH 12 for 24 h.

Film	WVTR (g m^{-2} day^{-1})	WVP (kg pa^{-1} m^{-1} s^{-1} $\times 10^{14}$)	PR (kg pa^{-1} m^{-2} s^{-1} $\times 10^{10}$)	pH 1 (%)	pH 7 (%)	pH 12 (%)
Blank	3685	–	–			
CH	918	9.09	9.09	101 ± 2	311 ± 2	96 ± 0.03
GG	977	4.25	9.67	51 ± 0.3	509 ± 13	(a)
CH$^{\text{into}}$GG	925	8.97	9.15	132 ± 7	224 ± 10	87 ± 4
GG$^{\text{into}}$CH	910	7.20	9.01	95 ± 1	493 ± 12	(b)
CH$^{\text{into}}$GG(pH)	974	10.60	9.64	52 ± 19	317 ± 11	104 ± 37
GG(pH)$^{\text{into}}$CH	932	9.86	9.23	115 ± 5	553 ± 2	66 ± 38
CH(pH)$^{\text{into}}$GG(pH)	1041	10.70	10.30	83 ± 15	392 ± 17	41 ± 6
GG(pH)$^{\text{into}}$CH(pH)	1056	8.78	10.50	145 ± 84	674 ± 22	53 ± 24

(a) film dissolved; (b) film split into separate layers.

3.3. *Polyelectrolyte complex films*

Several methods were assessed on their applicability for fabricating large free-standing PEC films. For example, dipping the single biopolymer films into a solution of oppositely charge biopolymer (similar to a dip-coating process) was not practical due to the volume of biopolymer solution required in the immersion bath (>2 L). The process was adapted by changing the orientation of the SB during dipping, i.e., from vertical to horizontal (Fig. 3). This arrangement requires an immersion bath volume of less than 200 mL. The optimum immersion time was determined as 30 s on each side.

We found that the order of addition had a profound effect on the PEC film composition and properties (Tables 1 and 2). Immersing a

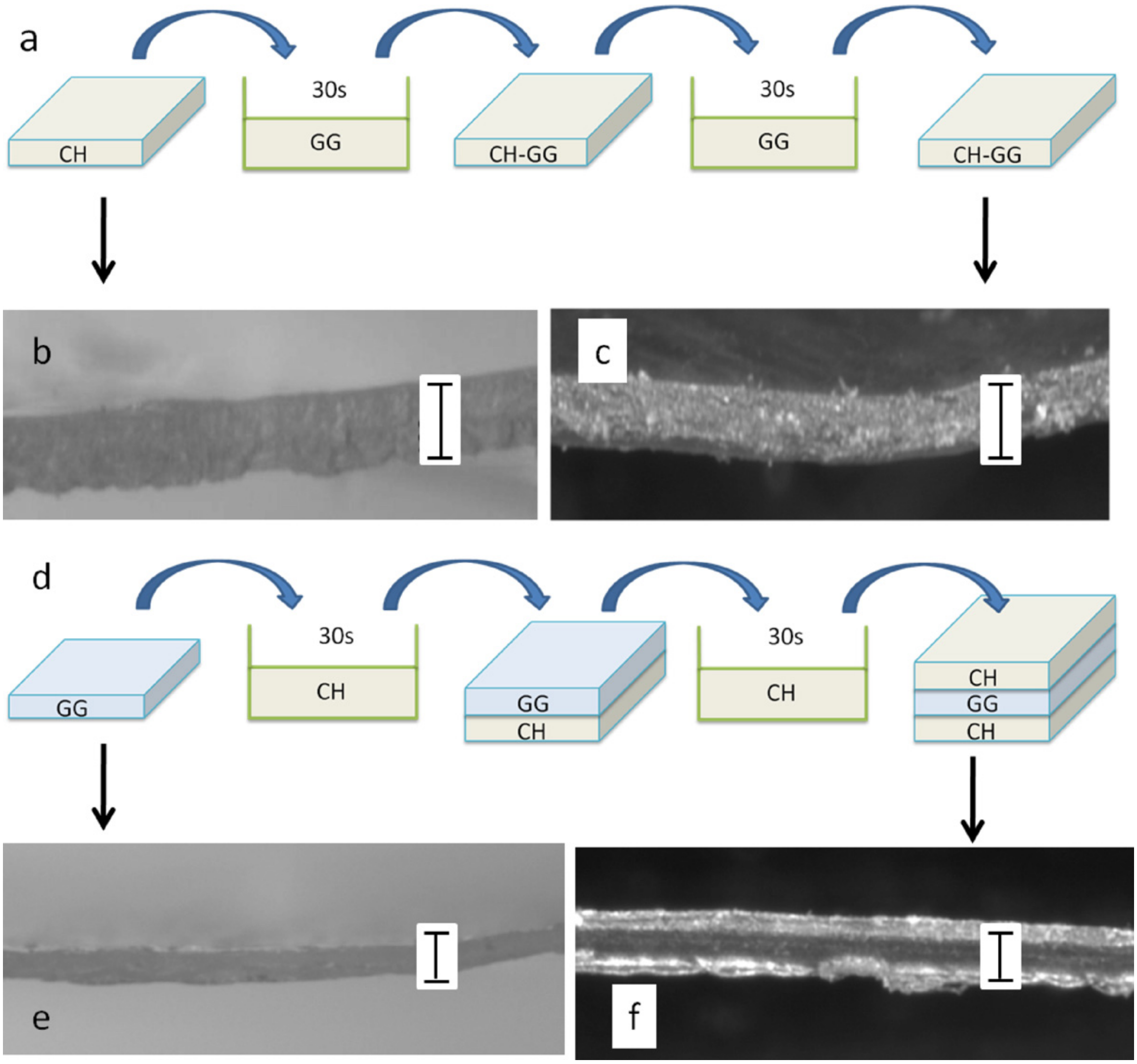

Fig. 3. Polyelectrolyte complex films. (a) Schematic of the preparation and composition of CH$^{\text{into}}$GG films. (b) and (c) Optical microscope images of the cross-section of CH and CH$^{\text{into}}$GG films, respectively. (d) Schematic of preparation and composition of GG$^{\text{into}}$CH films. (e) and (f) Optical microscope images of the cross-section of GG and GG$^{\text{into}}$CH films, respectively. Scale bars indicate 100 μm.

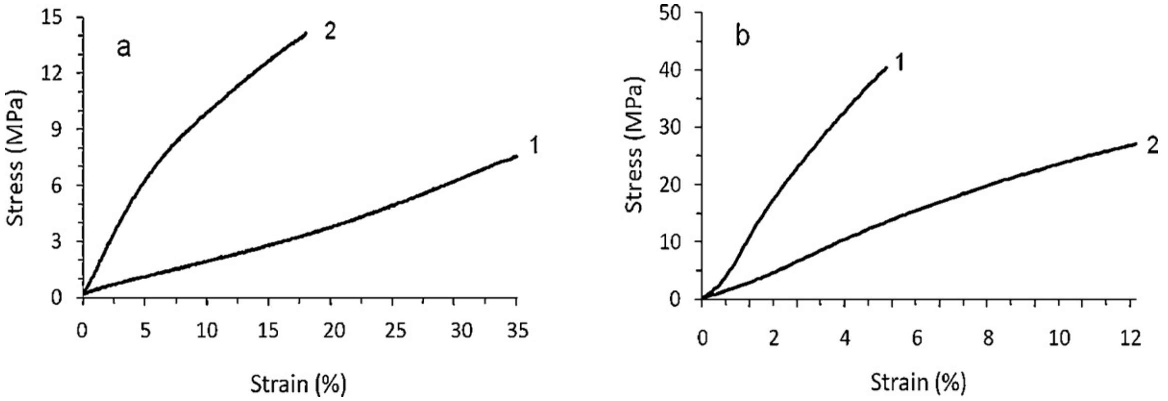

Fig. 4. Stress–strain curves for typical single-polymer and polyelectrolyte complex films. (a) CH (1) and CH^{into}GG (2) films, and (b) GG (1) and GG^{into}CH (2) films.

CH film into a GG solution, and vice versa are hereafter referred to as CH^{into}GG and GG^{into}CH, respectively, and immersing a CH film into a pH adjusted GG solution is referred to as CH^{into}GG(pH).

The mechanical characteristic of SB films change as a result of the PEC process. Immersing CH films into GG results in an improvement of toughness, Young's modulus and tensile strength values coupled with a decrease in extensibility compared to the chitosan SB film (Fig. 4). PEC films formed through immersion of GG films into CH results in the opposite trends: extensibility improves, but Young's modulus and tensile strength values all decrease compared to the corresponding SB film.

Changing the order of addition alters the biopolymer composition of the PEC films (Table 1). The difference in the composition of our PEC films is reflected in the mechanical properties (see Table 1). For example, changing the order of addition from CH^{into}GG to GG^{into}CH results in a 3.6 fold decrease in toughness, i.e., from 2.72 J g^{-1} to 0.76 J g^{-1}. A smaller change is observed for extensibility (a 1.6 fold reduction), while Young's modulus and tensile strength improved 1.7 and 2.7 times, respectively. In addition, CH^{into}GG films were less susceptible to swelling compared to GG^{into}CH (Fig. 5), while there is little difference in their water vapor transmission rates (WVTR) and permeance (Table 2). The range of WVTR values (910–1056 g m^{-2} day^{-1}) is similar to those exhibited by commercial dressings Duoderm® (886 ± 60 g m^{-2} day^{-1}) and Metoderm® (823 ± 45 g m^{-2} day^{-1}) (Wu, Fisher, Foo, Queen, & Gaylor, 1995).

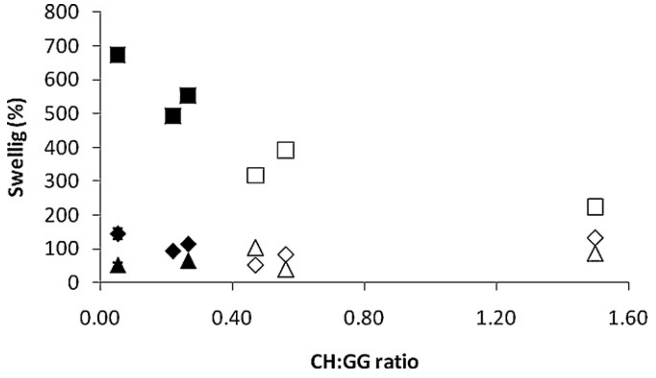

Fig. 5. Swelling of polyelectrolyte complex films as a function of CH:GG ratio after immersion for 24 h in solutions of pH 1 (diamonds), pH 7 (squares) and pH 12 (triangles). Films prepared by dipping GG into CH and CH into GG are indicated by filled and open symbols.

3.4. pH adjusted polyelectrolyte complex films

Using CH(pH) and GG(pH) solutions for either the formation of SB, or the immersion solution, or both, results in improvements in the mechanical characteristics. Tensile strength and density of PEC films prepared by immersing CH films into GG solutions increase linearly (Fig. 6) with increasing CH:GG ratio (Table 1). Although there is no linear relationship, it is clear that toughness and extensibility increase with increasing CH:GG ratio. In contrast, it is not possible to observe any trends for PEC films prepared by dipping GG films into CH solutions. Overall, it appears that the highest mechanical values are observed for PEC films with a CH:GG ratio of 0.47, or about twice as much CH as GG.

Let us now address why it is possible to observe these trends for PEC films prepared by immersing CH films into GG solutions, but not for GG films into CH solutions. Examining the cross-sections of these films shows that GG appears to diffuse into CH films (Fig. 3c), but CH does not diffuse into GG film (Fig. 3f). Optical microscopy suggests that the latter PEC films consist of an inner GG layer and two outer CH layers.

It is well-known that the multilayer structure of PEC films is affected by polymer charge density, polymer ionic strength, additive salt and type of polyion (Klitzing, Wong, Jaeger, & Steitz, 2004). There are a number of differences between CH and GG films and solutions: (i) CH has an intrinsic pK = 6.5 and requires acidic conditions to be soluble in water (Liu et al., 2005). GG has an intrinsic pK = 3.5 and is soluble in water (Sworn, Sanderson, & Gibson, 1995); (ii) the pH of CH films is always near neutral, regardless of the pH of solutions. For example, CH solutions of pH = 1.8, pH = 2.8 and pH = 5.4 results in films with pH values of 6.9, 6.7 and 7.4, respectively. GG solutions of pH = 5.6 and 12.0 result in films with pH values of 4.7 and 9.0, respectively; (iii) chain stiffness or the persistence length of GG is 9.4 nm at 40 °C (Takahashi et al., 2004). The temperature of our GG solutions during the formation is even higher (70 °C). Unlike CH, gellan gum chains convert from a double-helix structure to random coils upon heating, and will be in random coil conformation at 70 °C. The reported persistence length for CH (DD = 93%) is 81 nm at 25 °C (Cho, Heuzey, Bégin, & Carreau, 2006). In other words, it is suggested that CH chains are less flexible compared to GG chains. Therefore, it is likely that the difference in PEC composition shown in Table 1 may be attributed to the difference in persistence length, as well as being driven by alkalinity and acidity of films and solutions. For example, immersing an acidic GG film (pH 4.7) into an acidic CH solution (pH = 1.8, temperature = 25 °C) will impede diffusion of CH into GG due to electrostatic repulsion of the chains. This may result in the build up of a separate CH layers on the GG film as shown in Fig. 3f. It is also possible that the small persistence length of GG in the 70 °C solution may aid the diffusion leading to the composition shown in Fig. 3c.

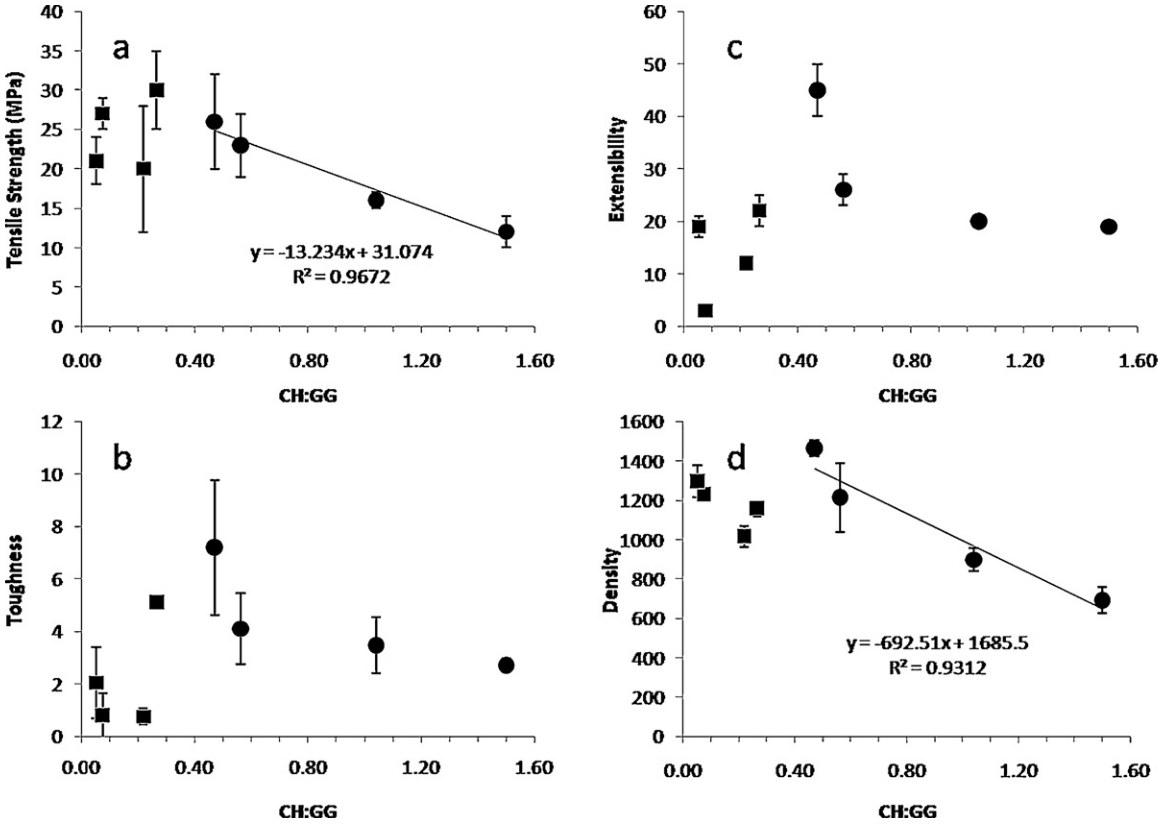

Fig. 6. Tensile strength (a), toughness (b), extensibility (c) and density (d) as a function of CH:GG ratio for polyelectrolyte complex film prepared by dipping CH into GG (circles) and GG into CH (squares).

4. Conclusion

PEC films were prepared by dipping free-standing films of GG into CH solution ($GG^{into}CH$) and CH into GG solution ($CH^{into}GG$). We show that the composition and mechanical characteristics depend on solution and film pH, as well as order of addition. Our results indicate that toughness, tensile strength, extensibility and density values of PEC films prepared by immersing CH films into GG solutions increase with increasing CH:GG ratio. For example, tensile strength increases from 12 MPa to 26 MPa for an increase in CH:GG ratio from 0.47 to 1.50. In contrast, it was not possible to observe similar trends for PEC films prepared by the reverse addition. We believe that the observed behaviour can be attributed to difference in their film compositions, i.e., $GG^{into}CH$ films consist of an inner GG layer and two outer CH layers, whereas for $CH^{into}GG$ films GG has diffused into the CH layer. This paper contributes to the understanding of polyelectrolyte complex materials.

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